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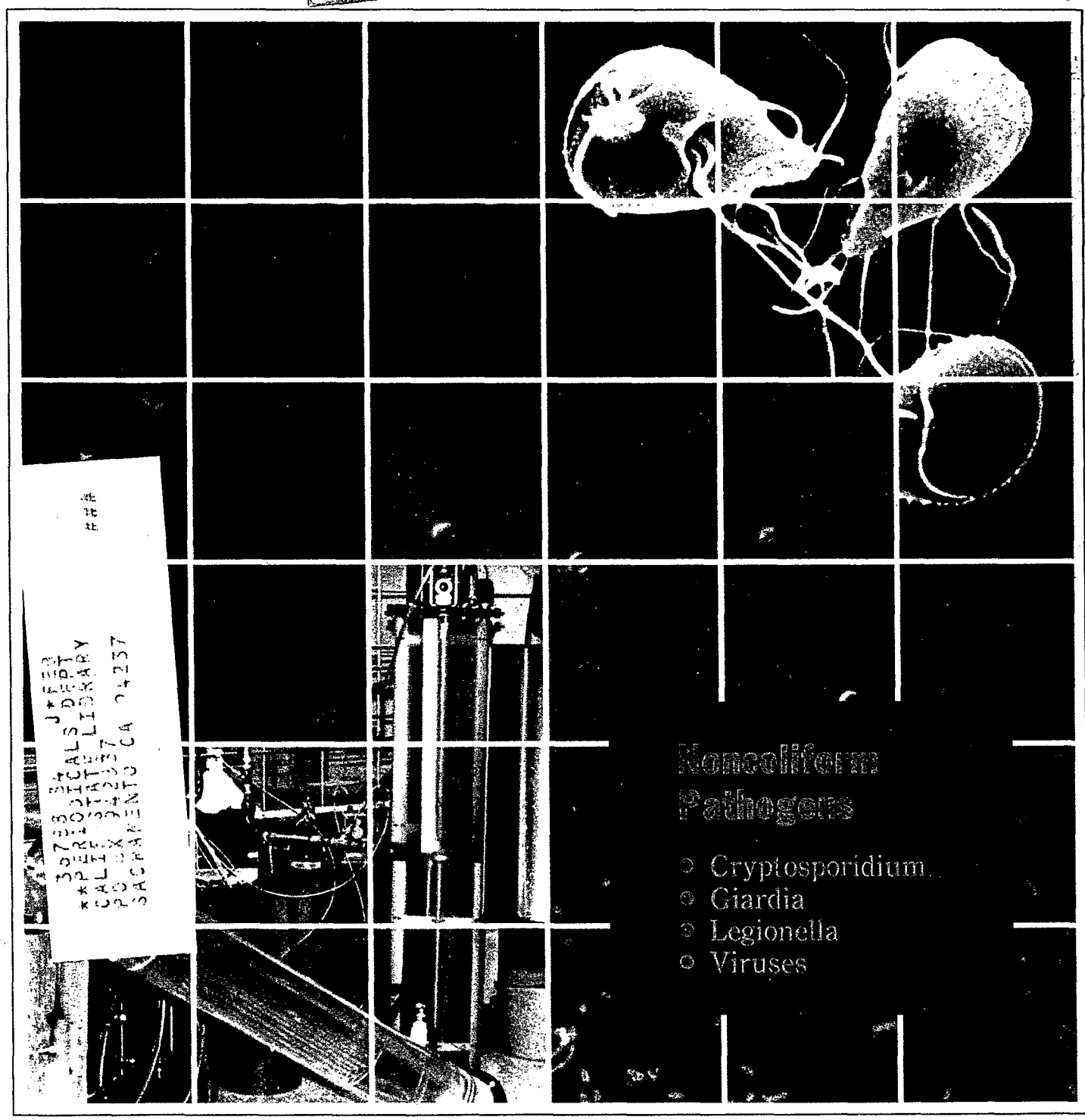

Noncoliform Pathogens

- Cryptosporidium
- Giardia
- Legionella
- Viruses

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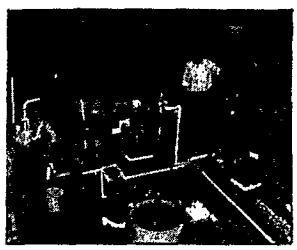
American Water Works Association
journal

Vol. 80, No. 2, February 1988



Noncoliform Pathogens: The various microorganisms that can threaten the safety of water supplies are discussed in this month's theme articles, beginning on **page 40.**

MANAGEMENT & OPERATIONS

RESEARCH & TECHNOLOGY

DEPARTMENTS

On the cover: *Photos of* Cryptosporidium *organisms (upper left and center) courtesy of Jerry E. Ongerth; remaining photos are (clockwise from upper right)* Giardia *cysts,* Legionella *organisms, and a pilot-plant used for virus removal*

• A blue dot precedes articles featuring the theme of this issue. The American Water Works Association disclaims responsibility for all information provided by the individual authors published in JOURNAL AWWA.

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Occurrence and Significance of Cryptosporidium in Water

Joan B. Rose

Three outbreaks of waterborne disease have been attributed to *Cryptosporidium*—two linked to drinking water and a third to surface water—yet the risk of waterborne disease is unknown because many factors may contribute to transmission. Of 107 surface water samples collected in six western states, 77 were positive for the presence of *Cryptosporidium* oocysts. A high count was found in raw sewage (1,732 oocysts/L), whereas low counts were found in waters without waste discharges (0.04 oocysts/L). *Cryptosporidium* has also been detected in drinking water. Little information is available, however, on oocyst survival in the environment or during sewage and drinking water treatment processes. Further research is necessary to define the variables that will influence the possible presence of infectious oocysts in water. It has been suggested that the epidemiology and transmission of *Cryptosporidium* are similar to *Giardia*. Based on environmental occurrence, the risk of *Cryptosporidium* transmission by the water route may be equal to or greater than that of *Giardia*.

Cryptosporidium has recently been recognized as an important microbial contaminant of water,[1] but its potential for transmission remains unknown. To adequately address the risk of waterborne transmission and waterborne disease, it is necessary to know (1) what the exposure through water is and (2) what disease manifestations occur at these levels of exposure.

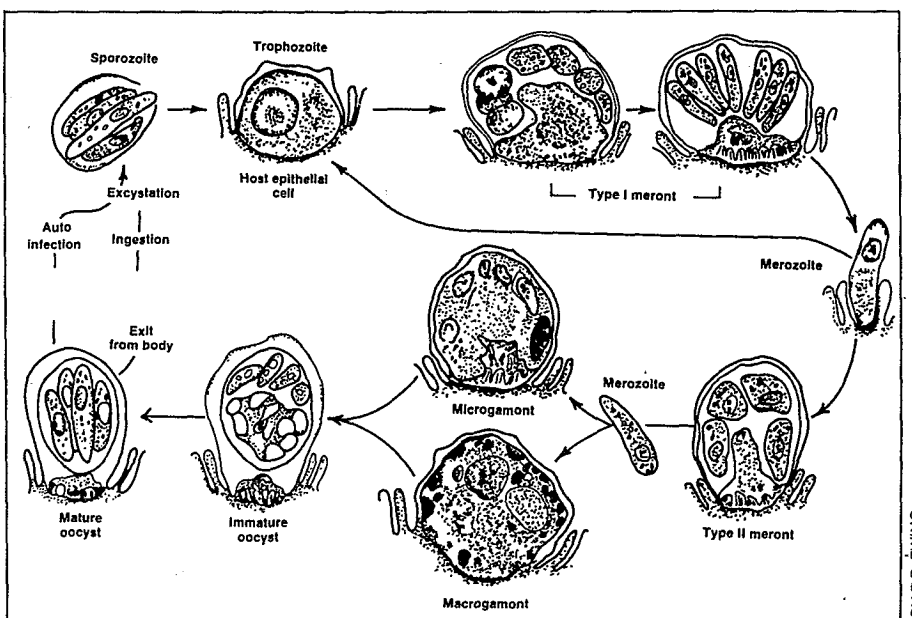
During the last five years, a tremendous amount of research has been published on *Cryptosporidium*. Relevant to the occurrence and significance of *Cryptosporidium* in water are (1) specific features of the organism that enhance its potential for transmission by water, (2) documentation of waterborne outbreaks, (3) methods for recovery and detection of *Cryptosporidium* in water, (4) data on occurrence in water, and (5) the ability of the organism to survive in water and water treatment processes. This article reviews literature that pertains to the transmission of *Cryptosporidium* by water and presents original data on the occurrence of *Cryptosporidium* in waters in the western United States.

Taxonomy and life cycle of *Cryptosporidium*

Cryptosporidium is taxonomically described as a coccidian protozoan. It has been placed in the phylum *Apicomplexa*, the order *Eucoccidioridia*, and the family *Cryptosporidiiae*.[2] *Cryptosporidium* was first described in 1907 by Tyzzer[3] as found in the gastric mucosa of mice. Many species were identified and differentiated taxonomically by the host from which the parasite was isolated.[4] It became apparent, however, that many mammalian isolates were able to cause infection in other mammals.

Levine[4] suggested four species designations corresponding to isolates from mammals, birds, reptiles, and fish. This classification has not been validated. Four species are recognized, however—*C. parvum* and *C. muris*,[5] found in mammals, and *C. baileyi* and *C. meleagridis*,[6] found in birds. *Cryptosporidium parvum* is the major species responsible for clinical illness in humans and animals.[7] A distinct isolate associated with adult cattle has recently been described by Anderson.[8] Little is known about its taxonomy or host specificity.

Two aspects of the taxonomy and life cycle of *Cryptosporidium* enhance the



DRAWING BY R.B. EWING

Figure 1. Life cycle of *Cryptosporidium* (*this drawing was originally published by the American Society for Microbiology in* Microbiological Reviews, *50:458 [1986]*)

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possibility of waterborne transmission. First, a single species may be responsible for much of the diarrheal illness in mammals, including humans. Second, an environmentally stable oocyst is excreted in the feces of infected individuals.

The organism appears to be ubiquitous. It is found in both domestic animals (cattle, sheep, swine, goats, dogs, and cats) and wild animals (deer, raccoon, foxes, coyotes, beavers, muskrats, rabbits, and squirrels).[7] Fayer and Ungar[9] have recently reviewed the cross-transmission studies on *Cryptosporidium*. Most significant are the data indicating that isolates from cats, cattle, and pigs are able to initiate infection in humans. Conversely, human isolates have produced infection in cats, dogs, cattle, goats, sheep, pigs, mice, and rats. Although *Cryptosporidium* infections may be common in bird populations, isolates from birds, chickens, and turkeys have not produced infections in mammals.[9] The *C. baileyi* found in birds may not be a major source for human infections, yet it is uncertain whether the same is true for *C. meleagridis*.

Cryptosporidium in humans, like many other important waterborne pathogens, completes its life cycle in the gastrointestinal tract. Because it is an obligate parasite, it can replicate only within its host. It is different from other enteric protozoa, such as *Giardia*, in morphology and life cycle. The life cycle of *C. parvum* begins with ingestion of the infectious stage—the oocyst—which releases four sporozoites after excystation. This stage initiates infection within the epithelial cells of the gastrointestinal tract. The sporozoite differentiates into the trophozoite, which undergoes asexual multiplication to form type I meronts and then merozoites, which may infect new host cells. Merozoites from type II meronts produce microgametocytes and macrogametocytes, which undergo sexual reproduction to form the oocyst, which is then excreted in the feces (Figure 1).[9] The oocysts are immediately infective upon excretion.

It appears that *C. parvum* is the major species of concern causing illness in both humans and animals.[7] Many mammals may serve as reservoirs of infection for humans.[7] This cross-species transmission increases the potential for waterborne disease because animals, in addition to humans, may also contaminate water sources. The most significant factor influencing the potential for waterborne transmission of *Cryptosporidium*, however, is the fecal-oral route of transmission from host to host by its environmentally stable oocyst.

Waterborne epidemiology of *Cryptosporidium*

Cryptosporidium is responsible for diarrheal disease and has been transmitted through the water route. It is not known how significant this route of transmission is in comparison to other possible paths, such as person to person. In more than 50 percent of the waterborne outbreaks in the United States, an etiologic agent was not identified.[10] *Cryptosporidium* may have been responsible for some of these outbreaks, yet may have gone unrecognized. Studies on waterborne outbreaks can provide valuable information on sources of contamination and the reliability of water treatment processes. In addition, information on disease in populations, other transmission routes, and infectious dose will help define the epidemiology of waterborne cryptosporidiosis.

Cryptosporidium was first recognized as a pathogen during an outbreak of diarrhea in a turkey flock in 1955,[11] after which it was identified as an infectious agent in cattle and sheep.[12-17] Serological studies indicate widespread infection in calves; neonates are most susceptible, with significant morbidity and mortality. Zoonotic transmission has been documented as previously described; however, this may not be a major transmission route.[9]

In humans, *Cryptosporidium* infections were first identified with immunocompromised individuals[18] and were brought to the attention of the medical community with the occurrence of acquired immune deficiency syndrome (AIDS).[19] *Cryptosporidium* has now been identified in diarrheal specimens from immunocompetent individuals ranging from a 0.12 to 23 percent prevalence.[9] In North America, *Cryptosporidium* has been found in 0.6 to 4.3 percent of the diarrheal cases. Children under two years of age may be the most susceptible, and *Cryptosporidium* has become problematic in day-care centers. Person-to-person transmission has been documented in day-care centers[20,21] and in hospitals[22,23] and may play a major role in the spread of disease.

Cryptosporidium is considered a primary pathogen. Current[7] has speculated, however, that the virulence of isolates may vary. That is, *Cryptosporidium* from different sources may vary in its capacity to cause infection and disease, depending on its virulence. Other investigators have also suggested a similar variation.[24,25] A second factor that will play a role in the initiation of disease is the infectious dose, which has not been well-defined for *Cryptosporidium*. Ernest et al[26] reported 22 percent infection with 100 oocyst inoculum in mice. Miller et al[27] presented data that indicated as few as 10 oocysts could initiate infection in primates. The ability of an organism to cause infection at low levels of exposure enhances the potential for waterborne transmission. Preliminary results suggest that *Cryptosporidium* may have a low infectious dose.

The role of *Cryptosporidium* as a cause of travelers' diarrhea has implicated transmission through contaminated water.[28,29] No definitive proof has been provided, yet *Cryptosporidium* infections were related to infections of other waterborne pathogens such as *Giardia*. Although speculative, illness in Caribbean tourists was associated statistically with the consumption of tap water.

Two waterborne outbreaks caused by the contamination of drinking water by *Cryptosporidium* have been documented. The first was a result of a sewage-contaminated well.[30] The well was chlorinated, although no residuals were

TABLE 1
Recovery efficiencies for Cryptosporidium *from various waters*

Study	Seed Volume L	Seed Level per L	Type of Water	Percent Recoveries	
				Ranges	Averages
Ongerth and Stibbs[39]	7–20	10^3–10^4	River	5–22	9.5
Musial et al[38]	378	0.2–2.6	Tap water	9–29	17.4
Rose et al[41]	378	10^3	Tap water	25–80	59
Rose et al[41]	378	0.4	Tap water	31–42	36
Rose et al[46]	378	10^4	Effluent	Not reported	20

TABLE 3
Cryptosporidium *oocyst concentrations in environmental samples*

State	Type of Water	Number of Samples	Number Positive	Oocyst/L Average
Arizona	Treated effluent	20	15	489
	Raw sewage	5	5	1,732
	Streams*	9	8	18
California	Streams	3	1	0.04
Colorado	River*	2	2	0.12
	Treated effluent	2	2	4.0
Oregon	Streams	6	5	0.05
Texas	Reservoirs*	6	6	1.1
	Raw sewage	6	4	4.1
Utah	Lakes and streams*	48	34	8.9
Total		107	82	

*Receiving sewage discharges

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TABLE 2

Summary of previous reports of Cryptosporidium *oocysts in sewage and various waters**

Water Source	Number of Samples	Range	Oocysts/L Average	Reference
Arizona				
Effluent	2	5-17	11†	40
Raw sewage	4	850-13,700	5,180	45
Effluent	11	4-3,960	1,063	45
Surface waters	6	0.8-5,800	1,920	45
Washington				
Surface waters	11	2-112	25†	39

*Arithmetic means
†Adjusted values based on recoveries

TABLE 4

Occurrence of Cryptosporidium *oocysts in various waters throughout the western United States*

Water Sampled	Number of Samples	Number of Samples Positive	Percent Positive	Oocysts/L*
Raw sewage	11	10	91	28.4
Treated sewage†	22	20	91	17
Reservoir, lake	32	24	75	0.91
Stream, river	58	45	77	0.94
Filtered drinking water	10	2	20	0.001
Nonfiltered drinking water	4	2	50	0.006

*Geometric means
†Activated sludge

reported. It was suggested that the contamination had percolated through the ground to the well. Oocysts were found in the stools of those with diarrhea, and the incidence of illness (117 cases) was 12 times greater than in the neighboring community. The Norwalk virus, which has been documented in previous waterborne disease outbreaks,[31] was also found in the diarrheal stools. There is some uncertainty regarding the amount of illness that could be attributed to *Cryptosporidium*. Because sewage was the source of the contamination, it might not be too surprising that multiple pathogens were implicated in the outbreak. No oocysts were recovered from the drinking source. However, methods for the recovery of oocysts from water were not well developed at that time.

The second outbreak occurred early in 1987, in Carrollton, Ga., a community of 16,000 people.[32] The increased incidence of student illness at a university clinic on January 20 first alerted the health department, and the Centers for Disease Control was brought in. It was determined that the illness was widespread throughout the community served by the public water system. Those individuals consuming well water had statistically less illness than those consuming city water. *Cryptosporidium* was identified as the etiologic agent through examination of diarrheal specimens.[33] The drinking water underwent conventional treatment (coagulation, sedimentation, filtration, disinfection) and met coliform (1 cfu/100 mL) and turbidity standards (1 ntu). Free chlorine residuals were as high as 1.5 mg/L at the treatment plant. After extensive sampling, oocysts were detected in the treated water.[33] Little published information that documents this outbreak is available. Future reports detailing the Carrollton outbreak of cryptosporidiosis will provide more insight into the waterborne transmission of *Cryptosporidium*.

In New Mexico, 78 cases of cryptosporidiosis were confirmed in 1986 through laboratory diagnosis of patients with diarrhea.[34] Seventy-four percent of the patients lived in the same county, and 32 of these individuals were considered household or day-care contacts of the 24 included in the study. Various risk factors were statistically evaluated to determine a possible source of infection. Drinking untreated surface water was found to be associated with illness. In addition, there may have been an increased risk of infection from swimming in surface waters. This is the first incident for which recreational exposure was suggested as a transmission route. Attendance at a day-care center in which other children were ill was also considered a risk factor, which confounded the analyses.

The previous discussion demonstrates the uncertainty in assessing the risk of waterborne cryptosporidiosis. Infectious dose and virulence variation are ill defined. Other transmission routes may conceal the role water has played as the source of infection. The greatest uncertainty is the contribution of *Cryptosporidium* to outbreaks of unknown etiology or to unrecognized, unreported outbreaks. Considering the difficulties with epidemiologic studies, even during outbreak situations these data probably should not be used to establish the significance of waterborne transmission of *Cryptosporidium*. Perhaps an alternative approach for the assessment of potential waterborne transmission is to evaluate the exposure by defining the occurrence of *Cryptosporidium* in water.

Recovery and detection methods for *Cryptosporidium* oocysts in water

To determine the occurrence of *Cryptosporidium* in environmental samples, the oocysts must be recovered and detected. Methods for environmental samples evolved partially from those used for *Giardia* and from those used in the clinical laboratory. This has included a combination of density gradients and flotations to concentrate and clarify the sample[35] and antibodies specific to the oocyst wall for detection using immunofluorescent techniques.[36-38]

In water samples, the concern is being able to detect low concentrations of oocysts in large volumes of water. Two similar systems have evolved that rely on concentration of the oocysts from water using filters. The method developed by Ongerth and Stibbs[39] employed 293-mm polycarbonate membrane filters. The method consists of first passing 20 L of water through a 5-μm prefilter. The oocysts were concentrated by subsequent passage of the filtrate through a 1.0-μm filter. The oocysts were recovered from the polycarbonate filter through the vibration of the inverted membrane in 200 mL of distilled water. The debris or sediment recovered was pelleted or concentrated using centrifugation, and the sample was clarified using a potassium citrate (1.19 g/mL) density gradient. The sample was filtered through cellulose nitrate membrane filters (1.2 μm) and stained with polyclonal antibody tagged with fluorescein isothiocyanate. The oocysts were detected using an epifluorescent microscope. The second method[40] used a 10-in. (250-mm) polypropylene cartridge filter (1.0-μm pore size) for concentrations of the oocysts from water. This system has an advantage over the polycarbonate system in that it can be easily transported to the sampling site, and large volumes of water (100-1,000 gal [378-3,785 L]) can be processed. A disadvantage is the elution procedure. The cartridge filter was processed with 6 L of a 0.1 percent distilled water solution* by backflushing, cutting apart, and washing the filter. Thus, it was necessary to concentrate 6 L of the eluent to a pellet using centrifugation, in contrast to approximately 300 mL when using the membrane filter method.

The cartridge filter system used a sucrose (1.24 g/mL) flotation to clarify the sample. High recoveries were achieved when 0.1 percent distilled water solution* and 1 percent sodium dodecyl sulfate were used with the sample. Oocysts were detected on a glass slide (or hemacytometer) using a monoclonal antibody and epifluorescent microscopy.

*Tween 80, J.T. Baker Chemical Co., Phillipsburg, N.J.

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Further development of the cartridge filter system by Rose et al[41] has included (1) decreasing the eluent volume to 2,700 mL, (2) improving clarification using sucrose at specific gravities of 1.24 and 1.17 g/mL, and (3) using a cellulose nitrate filtration membrane in conjunction with a monoclonal antibody for oocyst detection. Microscopic counts can be used to calculate the numbers in the equivalent volume passed through the membrane filter. This quantification may also be used to evaluate the efficiency of the methods in seeded experiments. Recovery rates for *Cryptosporidium* oocysts are influenced by the volume sampled, oocyst levels, and, most important, water quality. Large volumes, low levels of oocysts, and poor water quality (high turbidity, suspended solids, organic content) may decrease recoveries. Table 1 summarizes various method efficiencies. Average recoveries ranged from 9.5 percent in river water[39] to 59 percent in tap water.[41]

Levels (or counts) of oocysts found in water samples may be adjusted mathematically to reflect a more true concentration based on recovery method efficiencies. But this approach should be used cautiously. Unless seeded recoveries are determined concurrently with each sample tested, accurate determinations cannot be made. Recoveries vary even under controlled laboratory conditions, and characteristics of the water sample at the time of collection will influence this recovery rate.

In addition to poor recoveries, the current techniques have a number of other limitations. Oocysts excreted from birds, which may not be infectious for humans based on transmission studies between mammals and birds,[9] may be detected in some samples, depending on the antibody used. In all previously reported studies, no differentiation has been made between bird or mammalian oocysts.[36-38] The most significant limitation is probably the inability to determine oocyst viability. Viability of oocysts from fecal samples has been assessed through infection in a mouse model or through in vitro excystation.[26,42-44] Obviously, a bioassay system addresses infectivity but is expensive, less quantitative, and variable based on host susceptibility and infectious dose. Excystation is the process by which the oocyst opens up to release the sporozoites. This process may be induced in the laboratory. Excystation of an oocyst preparation may, however, decrease over time; the relationship between percent excystation and infectivity is undefined; and the counting of sporozoites and full and empty oocysts may be imprecise. Application of a bioassay or excystation procedure for determining the viability of oocysts recovered from environmental samples is currently impractical.

Environmental occurrence of *Cryptosporidium* in the western United States

Until 1985, little was known about the occurrence of *Cryptosporidium* in the environment. Because this protozoan is an enteric pathogen found in feces, it follows that the oocysts may be found in sewage and contaminated environments. Musial et al[40] estimated oocyst levels based on recoveries between 5 and 17/L in secondarily treated sewage. Madore et al[45] investigated select wastewater facilities and surface waters, many receiving wastes from dairy farms and beef-packaging plants. Waters receiving agricultural runoff from cattle pastures were also tested. High concentrations of oocysts were found in some sewage samples, averaging 5,180 oocysts/L in raw sewage and 1,063 oocysts/L in treated sewage. Surface waters varied between 0.8 and 5,800 oocysts/L. Ongerth and Stibbs[39] reported the presence of *Cryptosporidium* in several rivers in western Washington at levels between 2 and 112 oocysts/L (estimated from recoveries). A summary of these reports is presented in Table 2.

New information has come from more comprehensive investigations begun in 1986 on waters throughout the western United States for the occurrence of *Cryptosporidium*. Samples were collected using the cartridge filtration method. Filters were shipped to Arizona and processed using the methods previously described.[41] All data are reported at the levels observed in the samples without any adjustment because of recovery efficiencies.

Thus far, 107 samples have been collected from Arizona, California, Colorado, Oregon, Texas, and Utah. Various waters were sampled, including raw sewage, effluents (activated sludge), rivers, streams, lakes, and reservoirs. Many of the waters sampled were receiving treated sewage discharges. The results are summarized in Table 3. Of the 107 samples, 77 percent were positive for *Cryptosporidium*. Arithmetic means were calculated and ranged from 4.1 to 1,732 oocysts/L in treated sewage and from 0.04 to 18 oocysts/L in streams, reservoirs, and lakes. Streams sampled in Oregon and California had the lowest levels of contamination (0.05 and 0.04 oocysts/L, respectively), whereas waters sampled in Arizona and Utah were higher (18 and 19 oocysts/L, respectively). The higher concentrations were detected in surface waters receiving sewage discharges.

A wide variation in oocyst concentrations in the waters sampled was observed, with high levels detected occasionally in individual samples. This may reflect the variation in (1) collection of the grab samples, (2) recovery efficiencies influenced by the changes in water quality, or (3) contamination of the waters. To account for this variation, geometric means were calculated for these same samples by water types (Table 4). For raw and treated sewage, 91 percent of the samples were positive for *Cryptosporidium*, with average concentrations of 28.4 and 17 oocysts/L, respectively. The levels of oocysts detected in reservoirs or lakes and in streams or rivers were similar (0.91 and 0.94 oocysts/L, respectively), with 75 and 77 percent of the samples positive, respectively. The majority of these samples were receiving sewage effluents.

In addition, 14 drinking water samples were collected (Table 4). Of the 10 filtered samples, no oocysts were detected in waters receiving conventional treatment (coagulation, sedimentation, rapid sand filtration, disinfection). Oocysts were detected in one sample from drinking water after treatment by direct filtration. But the plant was not operating optimally because the mixers for the coagulants and ozonator were not functioning. One of two samples collected from waters receiving filtration without coagulation was positive for *Cryptosporidium* oocysts. Levels of oocysts in filtered drinking waters were low, averaging 0.001 oocysts/L (geometric mean). Two of four samples of unfiltered, chlorinated drinking water were positive for *Cryptosporidium*. Again, low levels were detected (0.006 oocysts/L).

Surface drinking water supplies in two distinct areas were sampled and tested for *Cryptosporidium*, *Giardia*, total coliform and fecal coliform bacteria, and turbidity. Six samples were collected from one area, area A, which included four reservoirs and two rivers. These were multiuse reservoirs and were open for recreational purposes. Three of the waters were receiving effluent discharges. Both waters were conventionally treated prior to distribution.

Six samples were collected from rivers in a second area, area B. These waters were in protected watersheds, closed to any public use, and were treated solely by disinfection. Samples were collected in October and November, at which time the weather was rainy in both areas A and B.

Table 5 contrasts area A with area B. Bacterial and turbidity concentrations were consistently 10 to 300 times higher in samples collected in area A. *Cryptosporidium* concentrations were also higher in area A, in which 100 percent of the samples were positive (averaging 0.99 oocysts/L), in comparison with 83 percent of the samples in area B, averaging 0.02 oocysts/L. Waterfowl were found at all the reservoirs in area A, however, and may have contributed to the contamination. Only one sample was positive for *Giardia* from each area. Concentrations were 0.29 and 0.006 cysts/L for

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TABLE 5

Levels of Cryptosporidium, Giardia, *bacteria, and turbidity in surface waters from areas A and B*

	Turbidity	Total* Coliforms Per 100 mL	Fecal* Coliforms Per 100 mL	Number Positive/ Number Collected	*Cryptosporidium* Oocysts/L†	Number Positive/ Number Collected	*Giardia* Cysts/L†
Area A	11.3	1,354	437	6/6	0.99	1/6	0.29
Range	2–33	200–24,000	110–8,000		0.19–3.0		
Area B	1.5	4.3	1.5	5/6	0.02	1/6	0.006
Range	0.08–4	0–30	0–10		0.01–0.13		

*Geometric means
†Arithmetic means

areas A and B, respectively. *Cryptosporidium* oocysts were detected more frequently and at higher concentrations than *Giardia*.

The poorer quality water in area A is reflected by the levels of bacteria, turbidity, and parasites. No statistical evaluations have been made because of the limited number of samples. It is, therefore, uncertain whether bacterial indicators or turbidity would be useful surrogates for the parasites.

The results presented on the investigation of waters in the western United States suggest that *Cryptosporidium* is ubiquitous in the water environment. Concentrations may vary 3 to 4 logarithms, depending on the types of wastes entering the water. *Cryptosporidium* can be found in source waters used for potable supplies and has occasionally been detected in treated drinking water. The significance of these findings is twofold: (1) drinking water treatment plants may be challenged with varying concentrations of *Cryptosporidium* oocysts in the source water, and (2) treatment processes should ensure adequate removal or inactivation of this potential pathogen.

Removal of *Cryptosporidium* by water treatment processes

Water treatment plays a significant role in the prevention of waterborne disease. It has long been recognized, however, that *Giardia* cysts are more resistant to disinfection than are bacteria or viruses. Therefore, in addition to disinfection, filtration has been recommended to ensure removal of parasite cysts. The oocysts of *Cryptosporidium* are smaller than the cysts of other protozoa and are the smallest of the coccidia, averaging 4 to 5 μm in diameter, with a spherical or slightly ovoid shape.[9] Removal and inactivation efficiencies for *Cryptosporidium* oocysts by filtration and disinfection are unknown, as these investigations are just beginning. Preliminary data are available that suggest the efficiency of some of these treatment processes.

Both secondary wastewater and wastewater after sand filtration have been examined for the occurrence of *Cryptosporidium*.[41] Based on average numbers of oocysts, an 87 percent removal could be estimated for the filtration step. A survey of a drinking water treatment plant employing filtration reported a large number of oocysts recovered off the filter backflush (2,906/L).[46] This indicates that *Cryptosporidium* will be concentrated on the filter. Comparison of oocyst levels in the source water to those in the finished water indicated a 91 percent reduction. These conclusions should be viewed cautiously because the data are limited and are not temporal. Also, both the wastewater and drinking water filtration systems were sand filters and were utilized without the addition of coagulants or polymers. A major outbreak of *Cryptosporidium* was, however, associated with a conventional rapid sand filtration plant that produced water meeting coliform and turbidity standards.[47] Various deficiencies may have been involved in the plant's failure to remove the oocysts. These included no mechanical agitation during the flocculation process and restarting filters without backwashing. Regardless of the operational problems, the plant was meeting current regulations. It may be that turbidity or coliform bacteria are not adequate indicators for *Cryptosporidium*. Currently, removal efficiencies of oocysts by various types of filtration are unknown. Operational variables that may influence oocyst breakthrough or surrogate indicators for efficient removals are undefined.

The efficacy of water disinfection is also unknown for *Cryptosporidium*. A few investigations have reported on the effectiveness of a number of disinfectants used in the laboratory.[48–50] Only one study included sodium hypochlorite, which has relevance to use in water disinfection. Campbell et al[50] reported on the effectiveness of a 3 percent solution of sodium hypochlorite (undiluted bleach). Newborn mice were used to ascertain oocyst viability. Oocysts in rat gut homogenates were mixed with an equal volume of the disinfectant and solutions were incubated at room temperature and 4°C for 2 and 18 h, respectively. The concentration of the disinfectant available initially and at the end of 2 or 18 h was not determined. It could be assumed that the gut homogenate had some chlorine demand. No quantitative determinations of inactivation were made. Although a 3- to 4-logarithm reduction may not be significant in clinical samples, it could be very significant in environmental samples. Nevertheless, infection was obtained in mice after 18 h exposure to undiluted bleach. Bacterial pathogens under similar conditions were readily destroyed. Although oocyst counts in this study were only slightly decreased after 18 h, the counting accuracy was not determined and no attempts were made to distinguish empty and full oocysts. *Cryptosporidium* may be extremely resistant to disinfection, but no assessment can be made on its resistance or susceptibility to water disinfectants until the experimental data on oocyst inactivation by failure to excyst or infect under controlled water disinfection conditions are reviewed. It may be stated that under conditions such as those observed during the outbreak in Carrollton, Ga., oocysts were able to survive routine disinfection practices, whereas coliform bacteria were not.

Potential for waterborne transmission of *Cryptosporidium*

There is no doubt that *Cryptosporidium* has the attributes of a waterborne pathogen. Yet the role water plays in its transmission is difficult to determine.

Although methods have evolved to study the occurrence of *Cryptosporidium* in water, recovery inefficiencies and an inability to determine viability are major limitations. The survival of oocysts in the environment and removals or inactivation by sewage- and drinking-water-treatment processes are unknown. Infectious dose and virulence variation are also undefined. Despite these limitations, it may be concluded that *Cryptosporidium* can be commonly found in water and high levels of contamination can occasionally occur. Water treatment operators currently have no basis for evaluating the adequacy of treatment processes for removal of *Cryptosporidium* oocysts.

A recent review by Current[7] concluded that: (1) the epidemiology and transmission of *Cryptosporidium* are similar to *Giardia* and (2) *Cryptosporidium* may now be included as a cause of human diarrheal illness and, in some cases, is the most common protozoan identified.

The importance of waterborne giardiasis in the United States has been recognized during the last decade. Based on environmental occurrence, the poten-

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tial for waterborne transmission of *Cryptosporidium* may be equivalent to or greater than that of *Giardia*. Further research on the biology and epidemiology of *Cryptosporidium*, as well as its occurrence and its survival in water, will further delineate the potential for waterborne cryptosporidiosis.

Acknowledgment

The author thanks Kelley Riley, Hamid Darbin, Lee Landeen, Yin Ping, and Charles P. Gerba, and Charles R. Sterling for supplying the monoclonal antibodies for *Cryptosporidium*. This work was supported by the US Environmental Protection Agency under grant CR-813536-01-0. This article has not been subject to agency review and therefore does not necessarily reflect the views of the agency; no official endorsement should be inferred.

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